Investigation of the Role of Congenital Cytomegalovirus Infection in the Etiology of Enlarged Vestibular Aqueducts

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Objective: To determine whether congenital cytomegalovirus (CMV) infection is an etiologic factor in the pathogenesis of enlarged vestibular aqueducts (EVA).

Design: Two different cohort studies.

Subjects: The study population comprised 19 subjects with a history of congenital CMV infection and sensorineural hearing loss (cohort 1); 39 subjects with nonsyndromic EVA and their unaffected mothers (cohort 2); and 16 control subjects with EVA associated with Pendred syndrome and bi-allelic mutations of the SLC26A4 gene and their unaffected mothers.

Results: In cohort 1, we detected EVA in 0 of 19 subjects with congenital CMV infection and sensorineural hearing loss. In cohort 2, anti-CMV serologic profiles were consistent with possible congenital CMV infection in 10 (26%) of 39 subjects with nonsyndromic EVA and 6 (38%) of 16 control subjects with Pendred syndrome ($P = .52$). These seroprevalence rates are similar to those expected in the general population (40%).

Conclusion: In spite of their auditory phenotypic similarities, congenital CMV infection is not a significant factor in the etiology of EVA.


Enlargement of the vestibular aqueduct (EVA) and its contents, the endolymphatic sac and duct, is the most common malformation of the inner ear associated with sensorineural hearing loss (SNHL). Enlargement of the vestibular aqueduct may be unilateral or bilateral; in bilateral cases, asymmetry of hearing loss severity, audiometric configuration, and the size of the vestibular aqueduct (VA) is common. Enlargement of the vestibular aqueduct may be associated with an incomplete cochlear partition in the same ear; this combination is termed Mondini malformation. Hearing loss in ears with EVA is usually prelingual or perilingual in onset and sensorineural or mixed and may be fluctuating or progressive.

A familial case of nonsyndromic EVA (NSEVA) provided the initial evidence for a genetic etiology of this disorder. Mutations of SLC26A4 (PDS), which encodes the anion transporter pendrin, were subsequently identified in patients with NSEVA as well as in a syndromic form of EVA called Pendred syndrome. SLC26A4 mutations are one of the most common genetic causes of SNHL in children, accounting for approximately 5% of childhood deafness among a wide variety of ethnic populations.

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Patients with bi-allelic SLC26A4 mutations have Pendred syndrome and manifest EVA with hearing loss and a thyroidal iodine organification defect. Patients with NSEVA may have 0 or 1 mutant SLC26A4 alleles. Although single mutant alleles of SLC26A4 seem to play an etiologic role in the development of some cases of NSEVA, they are not sufficient to cause NSEVA. Those NSEVA cases with 1 SLC26A4 mutation, if not all cases of NSEVA, appear to have a complex etiology that may include other genetic, epigenetic, or environmental factors. These factors might be less common in populations in whom EVA is most often associated with bi-allelic SLC26A4 mutations.
Several independent observations indicate that congenital infection with cytomegalovirus (CMV) may be an etiologic factor for EVA. Bauman et al \(^\text{10}\) described a bilateral Mondini malformation in a child with symmetric congenital CMV and SNHL. Radiologic examination of 6 affected ears from 4 other patients with symmetric congenital CMV and SNHL revealed 1 patient with bilateral SNHL and dysplastic inner ears, including a unilateral Mondini cochlear malformation, but normal VAs. The remainder of the affected ears had normal VAs. \(^\text{10}\) Temporal bone histopathologic studies of congenital CMV disease have identified CMV inclusion bodies in nonsensory epithelial cell populations, \(^\text{11,12}\) including the cells that express pendrin, \(^\text{13}\) providing further evidence of a potential pathogenic link between EVA and CMV.

Congenital CMV infection affects approximately 0.4% to 2.3% of live births in the United States. \(^\text{4,13}\) It usually goes unrecognized, since 90% of infants are asymptomatic at birth. \(^\text{16}\) Sensorineural hearing loss is the most common sequela, \(^\text{17}\) affecting 30% to 65% of those with symptomatic congenital CMV \(^\text{18,19}\) and 8% to 15% of those with asymptomatic congenital CMV infection. \(^\text{16}\) Congenital CMV infection is reported to account for up to 20% of childhood SNHL \(^\text{20}\) and is thus one of the most common single causes of SNHL in children.

There are striking similarities in the clinical presentation and course of EVA- and CMV-associated SNHL. The age of SNHL onset in patients with CMV ranges from 6 to 197 months, \(^\text{19}\) similar to that reported for EVA. \(^\text{2,4}\) Hearing loss in children with CMV is progressive in 50% and fluctuating in 23% of cases, \(^\text{19,21,22}\) which is similar to the natural history of SNHL associated with EVA. \(^\text{2,4}\) Furthermore, 33% to 52% of SNHL associated with congenital CMV is unilateral, whereas bilateral hearing loss is often asymmetric, \(^\text{19}\) again closely approximating the reported observations in patients with EVA. \(^\text{2,4}\) Finally, in both EVA- and CMV-associated SNHL, vestibular symptoms and signs are variable. \(^\text{2,4,22,24}\) These clinical similarities led us to investigate the possibility of an etiologic connection between congenital CMV infection and EVA. Because the diagnosis of congenital CMV infection requires viral culture within the first 3 weeks of life, and most cases of congenital CMV are asymptomatic, we used 2 distinct cohorts and 2 different study designs. We analyzed the seroprevalence of CMV exposure among subjects with EVA and, conversely, the prevalence of EVA among patients with congenital CMV and SNHL.

**METHODS**

**COHORT 1**

The first cohort comprised subjects recruited from 2 published, prospective, longitudinal studies of SNHL in children with culture-proven congenital CMV infection at the University of Alabama at Birmingham (UAB) \(^\text{18,19}\) and the Baylor College of Medicine (BCM). \(^\text{21}\) Parents or legal guardians of subjects with SNHL already enrolled in the UAB study were sent a letter from the UAB investigator (K.B.F.) inviting them to participate in the present study. Four subjects (age range, 12-14 years; mean, 14 years; median, 14 years) with asymptomatic congenital CMV infection were thus recruited and evaluated at the National Institutes of Health (NIH) Clinical Center (Bethesda, Md). This portion of the study was approved by institutional review boards at the NIH and UAB. Written informed consent was obtained for all subjects. The evaluation included a detailed medical history and physical examination (by S.P.P. or A.J.G.), family history, age-appropriate audiometry, review of past audiometry records, and computed tomography (CT) of the temporal bones. High-resolution imaging of the inner ears was performed using a fast induction employing steady state acquisition (FIESTA), a T2-weighted fast spin echo, or both on a 1.5-Tesla magnetic resonance imaging system. Enlargement of the VA was defined as a VA diameter exceeding 1.5 mm at the midpoint between the posterior cranial fossa and the vestibule of the inner ear or a grossly malformed overall morphology of the VA. \(^\text{1,9}\)

Authorization to review preexisting temporal bone CT studies and audiometric records was obtained by the BCM investigator (G.J.D.) from the parents of 15 subjects (age range at time of audiometry, 11 months–10 years; mean, 3 years; median, 6 years) with congenital CMV and SNHL. Most of the CT scans had been obtained as routine preoperative studies prior to cochlear implantation. We documented the age at time of CT scan, temporal bone findings on CT scan, age at time of audiometry, and laterality and degree of hearing loss. All copies of CT scans and audiometric records were returned to BCM after the completion of the study. This portion of the study was designated exempt from institutional review board review by the Office of Human Subjects Research (NIH).

**COHORT 2**

This portion of the study was approved by the joint institutional review board of the National Institute of Neurological Disorders and Stroke/National Institute on Deafness and Other Communication Disorders (NIH), and written informed consent was obtained for all participants. Thirty nine subjects (age range, 26 months–39 years; mean, 10 years; median, 7 years) with NSEVA and their unaffected mothers from 31 families were evaluated at the NIH Clinical Center. Their phenotypes and SLC26A4 genotypes have previously been reported. \(^\text{8}\) Sixteen patients (age range, 19 months–39 years; mean, 20 years; median, 12 years) with EVA of known etiology (Pendred syndrome: EVA with hearing loss and bi-allelic SLC26A4 mutations) were used as a control cohort. \(^\text{8}\) The evaluation included a detailed medical history and physical examination (by S.P.P. or A.J.G.), family history, age-appropriate audiometry, and review of past audiometry records.

Congenital CMV infection is rarely diagnosed at birth because it is usually asymptomatic, and it cannot be diagnosed by viral culture after 3 weeks of age. Therefore, serum anti-CMV IgM and IgG levels were measured in the NIH Clinical Center Department of Laboratory Medicine using the Wampole Laboratories (Princeton, NJ) Cytomegalovirus IgG enzyme-linked immunosorbent assay II (sensitivity, 96.4%; specificity, 93.9%) and IgM enzyme-linked immunosorbent assay II (sensitivity, 92.3%; specificity, 98.8%). Subjects were then assigned to 1 of 2 groups: (1) those in whom congenital CMV infection could not be ruled out, or (2) those in whom congenital CMV infection could be ruled out. The presence of anti-CMV IgM or IgG was considered evidence of previous infection with CMV. Conversely, CMV-specific IgG persists through the lifetime of normal hosts, and failure to detect it in commercially available assays rules out previous infection and congenital infection. \(^\text{17}\)

Therefore, congenital CMV infection could be ruled out in subjects with no evidence of previous CMV exposure or those in whom their mother had no evidence of pre-
virus or in subjects with syndromic EVA. However, we found that two thirds of our NSEVA cohort had findings inconsistent with congenital CMV infection. These proportions do not differ markedly from those that might be expected in the general US population (approximately 40%) and from those in a group of patients with EVA caused by bi-allelic SLC26A4 mutations. It is likely that most of our EVA subjects with serologic evidence of past CMV infection had postnatal exposure that is etiologically unrelated to their EVA and SNHL.

While our cohorts are small and our data cannot rule out the possibility that a small number of cases of EVA may be related to CMV, many cases of NSEVA clearly occur in the absence of congenital CMV infection. Therefore, some cofactor(s) other than CMV must contribute to the pathogenesis of EVA. Conversely, it appears that most patients with congenital CMV infection and SNHL do not have EVA, in spite of their auditory phenotypic similarities. Nevertheless, it remains possible that these similarities reflect a common pathogenic pathway involving loss of pendrin function in nonsensory epithelia that can be infected with CMV. Perhaps endolymphatic system and vestibular aqueduct morphogenesis are sufficiently completed prior to a putative loss of pendrin function in congenital CMV infection, or residual levels of pendrin function are sufficient to preserve morphogenesis but not auditory function.

It is possible that the association of EVA with congenital CMV and SNHL in the case reported by Bauman et al may have been coincidental. Approximately 0.4% to 2.3% of patients with NSEVA could be expected to have had congenital CMV infection if the prevalence is no different in this group from that in the general population, and 10% of these might be expected to have symptomatic CMV disease. Alternatively, the patient reported by Bauman et al may not have met formal criteria for EVA.

The lack of a strong etiologic association of congenital CMV infection with EVA has implications for the evaluation of children with SNHL, especially hearing loss with features that are characteristic of EVA or congenital CMV infection such as delayed onset, fluctuation, progression, asymmetry, or unilaterality. All children with SNHL, including children with culture-documented congenital CMV infection, should undergo ophthalmologic screening and receive routine audiologic monitoring. The diagnostic yield of an extensive workup, including temporal bone CT imaging or genetic testing, in children with culture-documented congenital CMV infection and SNHL is unknown. The yield should be increased in cases with a family history of SNHL or auditory features that are commonly associated with EVA but not CMV-associated hearing loss such as significant air-bone gaps extending into middle frequencies in the setting of normal middle ear function or sudden drops in hearing following minor head trauma or barotrauma. Similarly, the diagnostic yield of other evaluations such as GJB2 mutation testing may be higher in patients with a positive family history or with auditory features that are more suggestive of autosomal recessive nonsyndromic deafness DFNB1 or other genetic causes of nonsyndromic deafness. Such features include congenital or prelingual onset, symmetry, and lack

### RESULTS

#### COHORT 1

We detected EVA in 0 of 19 patients (34 ears) with congenital CMV infection and SNHL. The ages and audiometric findings in this cohort are given in Table 1.

#### COHORT 2

Ten subjects had serologic profiles consistent with possible congenital CMV infection, and 29 subjects had serologic profiles inconsistent with congenital CMV infection (Table 2). In the control group of patients with Pendred syndrome caused by bi-allelic SLC26A4 mutations, 6 subjects had serologic profiles consistent with possible congenital CMV infection and 10 subjects had serologic profiles inconsistent with congenital CMV infection. Results from a Fisher exact test showed no significant difference in these proportions between the subjects with NSEVA and control subjects with syndromic EVA (P = .52).

### COMMENT

Our data indicate that congenital CMV infection is not a common cause of NSEVA. If CMV were a common etiologic cofactor in the pathogenesis of EVA, we would expect to see a higher rate of CMV seropositivity in subjects with NSEVA compared with the general population.

<p>| Table 1. Cohort 1: Subjects With Congenital Cytomegalovirus Infection and Hearing Loss |
|---------------------------------|-------------------------------------|</p>
<table>
<thead>
<tr>
<th>Patient Age, y</th>
<th>Hearing Loss†</th>
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<td>30 mo</td>
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<tr>
<td>7</td>
<td>B profound</td>
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<td>8</td>
<td>B severe-profound</td>
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<td>13 mo</td>
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<td>6</td>
<td>B profound</td>
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<td>11 mo</td>
<td>B profound</td>
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<td>3</td>
<td>L severe; R profound</td>
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<td>6</td>
<td>B profound</td>
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<td>14</td>
<td>B severe-profound</td>
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Abbreviations: B, bilateral; L, left; R, right.
*Ages younger than 3 years are reported in months.
†Based on 0.5/1/2/4-kHz pure-tone average.

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of significant SNHL progression. Future studies might clarify if the potential for more accurate information on diagnosis, associated syndromic abnormalities, prognosis, rehabilitation, or recurrence risk justifies the added expense.

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REFERENCES


